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Methyl Mercaptan (CAS 74-93-1)
Methyl Mercaptide (CAS 5188-07-8)

**High Production Volume Challenge Program
Test Plan**

Submitted By:

Mercaptans/Thiols Council
941 Rhonda Place S.E.
Leesburg, VA 20175
(703) 669-5688

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I. PLAIN LANGUAGE SUMMARY

The Mercaptans/Thiols Council (MTC) has volunteered to provide basic hazard information for Methyl Mercaptan (MeSH), CAS Number 74-93-1, and Methyl Mercaptide (NaMeSH), CAS Number 5188-07-8, as part of the United States Environmental Protection Agency's High Production Volume Chemical Challenge Program (HPV Challenge).

MeSH and NaMeSH should be considered analogs because NaMeSH is the salt of MeSH. NaMeSH will be used for the proposed testing because it is converted to MeSH and it is safer and easier to handle.

In consideration of animal welfare concerns to minimize the use of animals in the testing of chemicals, the MTC has conducted a thorough literature search for all available data, published and unpublished. It has also performed an analysis of the adequacy of the existing data. In addition, we have used structure-activity relationship information to fill certain data gaps.

MeSH and NaMeSH have, or are expected to have, similar health and environmental hazard profiles. The metabolism and toxicological properties of hydrogen sulfide (H₂S) are similar to MeSH. For reproductive and developmental toxicity, surrogate data from a H₂S study is included.

Sufficient data are available to assess the physical/chemical and human health endpoints included in the HPV Challenge. Computer modeling or testing is proposed to better evaluate the environmental fate and aquatic toxicity of these chemicals. The following studies are being proposed to better assess the ecotoxicity and environmental fate of MeSH and NaMeSH: acute fish toxicity and acute algae inhibition. Computer modeling will be used to evaluate the photodegradation and transport in the environment (fugacity) for MeSH and NaMeSH.

CAS#	Name	Acronym	Status
74-93-1	Methyl Mercaptan	MeSH	Sponsored in HPV program
5188-07-8	Sodium Mercaptide	NaMeSH	Sponsored in HPV program
7783-06-4	Hydrogen Sulfide	H ₂ S	Not part of the HPV program but data to fill data gaps

II. MEMBER COMPANIES OF THE MERCAPTANS/THIOLS COUNCIL

- ATOFINA Chemicals, Inc (formerly Elf Atochem North America, Inc)
- Bayer Corporation*
- Chevron Phillips Chemical Company LP (formerly Phillips Chemical Company, Phillips Petroleum Company)
- Natural Gas Odorizing, Inc., a wholly owned subsidiary of Occidental Chemical Corporation*

* Members not producing/importing MeSH and NaMeSH

III. INTRODUCTION

The Mercaptans/Thiols Council (MTC) has volunteered to participate in the Environmental Protection Agency's High Production Volume Challenge Program (HPV Challenge) to assess the health and environmental hazards, including selected physical chemical characteristics of methyl mercaptan (MeSH) and methyl mercaptide (NaMeSH). These two chemicals should be considered analogs according to an EPA guidance document, 1999.

This document includes justification for considering MeSH and NaMeSH as analogs to be used interchangeably to assess the data endpoints included in the HPV Challenge. NaMeSH is the sodium salt of MeSH, which is formed when MeSH is added to a sodium hydroxide solution. NaMeSH is expected to be converted to MeSH because the pH values normally found in biological and environmental systems are below the pKa (10.7). Thus, toxicological information obtained for NaMeSH in these studies is equivalent to that of MeSH.

Our objective in this submission is to evaluate the available data and determine what additional data are needed to adequately characterize the human health and environmental hazards of MeSH and NaMeSH (Table 1). An evaluation of the available data for both MeSH and NaMeSH and proposed test plan are included. In addition, available information for hydrogen sulfide (H₂S) is included as surrogate data to complete a MeSH and NaMeSH health hazard assessment.

Based on our review of available data, MTC proposes to conduct acute fish toxicity and algae inhibition studies with NaMeSH. In addition, appropriate computer models will be used to calculate data for selected environmental fate and physical/chemical endpoints of MeSH and NaMeSH as suggested in EPA guidance documents. Substantial and scientifically defensible similarities between MeSH/NaMeSH and H₂S toxicological data provide the scientific basis to justify the use of reproductive and developmental H₂S toxicological information as surrogate data for MeSH and NaMeSH. Robust summaries of selected studies for MeSH and NaMeSH, as well as, the relevant robust summaries for H₂S are included in Appendices I, II and III.

TABLE 1: Matrix of Available Data and Proposed Data Development for Methyl Mercaptan (MeSH) and Methyl Mercaptide (NaMeSH)

EPA HPV Challenge Endpoint	Results of Data Review/Proposed Data Development
Physicochemical Properties	Calculate / Identify Existing Data
Biodegradation	Adequate Data / No Testing
Photodegradation	Calculation
Hydrolysis	Adequate Data/No Testing
Fugacity	Calculation
Acute Fish Toxicity	Testing Proposed
Acute Daphnia Toxicity	Adequate Data/ No Testing
Algae Toxicity	Testing Proposed
Acute Oral Toxicity	Adequate Data / No Testing
Acute Inhalation Toxicity	Adequate Data / No Testing
Acute Dermal Toxicity	Adequate Data / No Testing
Repeated Dose Toxicity	Adequate Data / No Testing
Genotoxicity, In Vitro	Adequate Data / No Testing
Genotoxicity, In Vivo	Adequate Data / No Testing
Reproductive/Developmental Toxicity	Adequate Data (H ₂ S data) / No testing

IV. USES OF METHYL MERCAPTAN AND METHYL MERCAPTIDE

Methyl mercaptan is used as a gas odorant, catalyst, intermediate in manufacturing jet fuels and in the synthesis of methionine, as well as, the manufacture of some pesticides and fungicides.

NaMeSH is an easier to handle, pumpable solution, which reduces the safety hazards of a toxic gas under pressure, associated with MeSH. Most applications for NaMeSH are for smaller reactions where the high value of the end product can justify the higher cost of using the more costly raw material. In all of these reactions, the MeSH moiety is released from the high pH solution by lowering the pH to be reacted with another chemical species, or is reacted directly from the NaMeSH.

V. ANALOG CHARACTERIZATION

According to the EPA, chemicals and their corresponding salts may be considered analogs (EPA guidance document, 1999). NaMeSH is the salt of MeSH and is produced by bubbling MeSH through aqueous sodium hydroxide. The value for the pK_a of NaMeSH in water at 25°C is 10.70 (Lange, 1985). At a temperature of 25°C and a pH of 10.7, there is an equilibrium of 50% MeSH and 50% NaMeSH dissolved in the water. The higher the pH, the more the equilibrium is shifted to the salt mercaptide

moiety. In other words, as a pH of 14 is approached, the solution moves toward being mostly NaMeSH. Conversely, the lower the pH, the more the equilibrium shifts to the pure MeSH being the chemical species in the aqueous phase.

For each drop in pH unit of 1.0, there is a corresponding drop by a factor of ten in the concentration of the NaMeSH in the aqueous state or conversely an increase in the MeSH. At a pH of 8.7, the ratio has been changed to roughly 1:100 (NaMeSH to MeSH), which means that the important chemical species present in solution is now the MeSH. The pH of a biological system is around 7.0 to 7.4 (CRC Handbook, 1995); therefore, the ratio of NaMeSH to MeSH is at least 1:1000. Thus, in biological systems, NaMeSH will be converted to MeSH.

Since all testing will be conducted below the pKa of NaMeSH, we propose that NaMeSH and MeSH be considered as analogs for assessing the health and environmental endpoints outlined in the HPV Challenge Program.

VI. EVALUATION OF PHYSICOCHEMICAL DATA

The physicochemical endpoints for the HPV Challenge include: melting point, boiling point, vapor pressure, water solubility, and octanol/water partition coefficient (K_{ow}). The physical/chemical data are detailed in the IUCLID dossiers (Appendices I and II). The data provided below are measured, reported in handbooks, or calculated using the EPIWIN[®] computer model. This model is discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program"(1999) and will be used to calculate physicochemical data for some of the endpoints where data are not available. The water solubility of NaMeSH will be confirmed in the aquatic toxicity studies proposed in Section VIII.

TABLE 2: Summary of Physical/Chemical Characteristics of MeSH and NaMeSH

	MeSH (gas)	NaMeSH (liquid)
CAS#	74-93-1	5188-07-8
Melting Point (°C)	-123	210 (crystallization temp 55)
Boiling Point (°C)	5.96 under 1 atm	69
Vapor Pressure (mm Hg@25°C)	1.51E+3	1.08E-6
Water solubility (mg/l)	23300	1000000
Octanol/Water Partition Coefficient (Kow)	0.78	-2.3

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

VII. EVALUATION OF ENVIRONMENTAL FATE DATA AND PROPOSED TESTING

Environmental fate endpoints for the HPV Challenge include: biodegradation, photodegradation, hydrolysis, and fugacity. Robust summaries on available environmental fate data, prepared in accordance with criteria outlined in the HPV Challenge, are provided in Appendices I and II.

A. Biodegradation

Biodegradation data, available for both products in this category, show that these products are readily biodegradable. NaMeSH is readily biodegradable in an OECD 301d "Ready biodegradability: Closed bottle test" (Elf Atochem, 1995). The overwhelming data indicate MeSH is biodegradable (Appendix I). The available data are sufficient to assess the biodegradability of MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

B. Photodegradation – Atmospheric Oxidation

Photodegradation can be measured (OECD test guideline 113) or estimated using models accepted by the US EPA and other authorities. An estimation method accepted by the US EPA includes the calculation of atmospheric oxidation potential (AOP). Atmospheric oxidation as a result of hydroxyl radical attack is not direct photochemical degradation, but rather indirect degradation. AOPs can be calculated using a computer model. The computer program AOPWIN (Atmospheric Oxidation Program for Microsoft Windows), used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances), calculates a chemical half-life based on an overall OH^\cdot reaction rate constant, a 12-hour day, and a given OH^\cdot concentration. AOPWIN will be used to estimate photodegradation for MeSH and NaMeSH.

SUMMARY: Photodegradation estimates (AOPWIN model) are proposed for MeSH and NaMeSH.

C. Hydrolysis

Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include: alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Stability in water can be measured (OECD test guideline 111) or estimated using models (HYDROWIN) accepted by the US EPA and other authorities. HYDROWIN cannot estimate the hydrolysis for structures such as MeSH and NaMeSH. Measuring hydrolysis at the specific pHs cited in OECD 111 guideline would result in the conversion of NaMeSH to MeSH.

In addition, MeSH and NaMeSH do not contain hydrolyzable moieties. Analytical measurement of MeSH in the acute daphnia study indicates MeSH is stable. The available data are sufficient to assess the hydrolysis of MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

D. Chemical Transport and Distribution In The Environment (Fugacity Modeling)

Chemical transport can be assessed using a Level III fugacity model to determine the relative distribution of chemicals between selected environmental compartments such as air, soil, sediment, and water. A widely used fugacity model is the Equilibrium Criterion Model that is included in the EPIWIN version 3.02 software currently used by EPA to evaluate new chemicals.

SUMMARY: An estimation from a Level III fugacity model is proposed to assess the transport and distribution of MeSH and NaMeSH in the environment.

VIII. EVALUATION OF ECOTOXICITY DATA AND PROPOSED TESTING

Aquatic toxicity endpoints for the HPV Challenge include: acute toxicity to freshwater fish, invertebrates, and freshwater algae. Based on the available data, MeSH and NaMeSH are expected to be toxic to aquatic organisms. For proposed testing, NaMesh will be used since it is safer and easier to handle and will convert to MeSH. Robust summaries on available ecotoxicology data, prepared in accordance with criteria outlined in the HPV Challenge, are provided in Appendices I and II.

A. Acute Fish Toxicity

In a 1952 study, MeSH is toxic to a variety of fish species with lethality occurring at concentrations between 0.5 – 1.75 ppm. Similar toxicity to fish is expected for NaMeSH. In order to adequately compare the data, an acute fish toxicity study (OECD 203) is proposed for NaMeSH.

SUMMARY: An acute fish toxicity study (OECD 203) with NaMeSH is proposed.

B. Acute Daphnia Toxicity

Based on a recent guideline (OECD 202 Part 1) study, NaMeSH is toxic to daphnia. The EC₅₀ (concentration immobilizing 50 percent of daphnia) after 48-hour exposure was between 1.32 – 2.46 mg/l. In fact, MeSH was the measured moiety in this study providing further support for the use of NaMeSH data to assess MeSH aquatic hazards. Similar results are expected for MeSH.

Sufficient data are available to assess the hazards of MeSH and NaMeSH to daphnia.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

C. Acute Algae Inhibition

Available data indicate MeSH and NaMeSH are toxic to fish and invertebrates. However, no data are available to assess the effects of MeSH and NaMeSH on algae (often more sensitive to toxic insult than fish and daphnia). Therefore, an algae inhibition study (OECD guideline 201) is proposed for NaMeSH.

SUMMARY: An algal inhibition study (OECD 201) is proposed for NaMeSH.

IX. EVALUATION OF HEALTH EFFECTS DATA AND PROPOSED TESTING

The mammalian toxicity endpoints for the HPV Challenge Program include: acute toxicity, repeat dose toxicity, genetic toxicity (including point mutations and chromosomal effects), and reproductive/developmental toxicity. Robust summaries on available toxicology data, prepared in accordance with criteria outlined in the HPV Challenge Program guidance documents, are provided in Appendices I and II.

A. Acute Toxicity

Acute toxicity studies have been conducted on MeSH and NaMeSH, which are summarized in Table 3. Inhalation exposure was used to assess the acute toxicity for MeSH, and oral and dermal exposure were used to evaluate the acute toxicity of NaMeSH. Regardless of the route of exposure, the toxicity was similar with CNS and respiratory depression, the common symptoms noted after high dose acute exposure. The available data are sufficient to assess hazards from acute exposure to MeSH and NaMeSH.

TABLE 3: Acute Toxicity of MeSH and NaMeSH

	MeSH (gas)	NaMeSH (liquid)
Inhalation LC ₅₀ (ppm)	675 ¹	No data
Oral LD ₅₀ (mg/kg)	NA	109 ²
Dermal LD ₅₀ (mg/kg)	NA	>84 ³

NA = not applicable

1 Tansy et al, 1981

2 Elf Atochem, 1989

3 Elf Atochem, 1994

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

B. Repeat Dose Toxicity

A 90-day repeat dose inhalation toxicity study has been conducted on MeSH. Male Sprague-Dawley rats were exposed to 2, 17 and 57 ppm for 7 hr/day, 5-days/week. Terminal body weights, organ weights, oxygen consumption, systolic blood pressure, intestinal transit activities, SMA 12/60 Analysis¹, and histopathology of selected organs were evaluated. No mortality was observed in any of the sham or exposed population of rats. The high dose group had a statistically significant decrease in body weight gain. The authors state that although some average organ weights were significantly different from corresponding sham values, there were no obvious dose-related trends (Tansy et al, 1981).

According to the literature, mercaptans are known to be potent ocular and dermal irritants in workers at levels exceeding acceptable workplace exposure standards. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV), 8-hour time weighted average (TWA), of 0.5 ppm for MeSH (ACGIH, 2001). Due to the intense odor and irritation of MeSH and NaMeSH, workers would limit exposure to levels above the TLV. Sufficient data are available to assess the hazards associated with repeated exposure to MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

C. Genetic Toxicity

1. Point Mutation

NaMeSH is not mutagenic in bacterial mutagenicity assays (Elf Atochem, 1992). The available data are sufficient to assess the mutagenic hazards of MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

2. Chromosomal Aberrations

MeSH was negative in a mouse micronucleus assay (Elf Atochem, 1997). NaMeSH was negative in a mouse micronucleus assay (Elf Atochem, 1999). The available data are sufficient to assess the chromosomal effects of MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

¹ SMA 12/60 Analysis included 13 different blood serum components; total protein, albumin, Ca⁺⁺, inorganic phosphorus, cholesterol, BUN, Uric acid, total bilirubin, alkaline phosphatase, LDH, SGPT, SGOT, glucose. No blood cell count analysis was performed.

D. Reproductive and Developmental Toxicity

No reproductive or developmental toxicity studies are available on MeSH or NaMeSH. Repeat dose studies conducted on MeSH did not evaluate the reproductive organs (Tansy et al, 1981).

A recent reproductive/developmental toxicity study of H₂S is included as surrogate data to assess the reproductive and developmental toxicity of MeSH and NaMeSH. Robust Summaries for select H₂S studies are included in Appendix III.

1. Rationale for Using H₂S Data

a. Similar Physical/Chemical Characteristics

The physical properties provided in Table 4 support the argument that H₂S and MeSH are similar. NaMeSH, a liquid, is different from the other two gases; however, it is a liquid, salt analog of MeSH in biological systems.

TABLE 4: Comparison of Physical/Chemical Properties

Chemical Name	Hydrogen sulfide	Methyl Mercaptan	Methyl Mercaptide
Acronym	H ₂ S	MeSH	NaMeSH
CAS #	7783-06-4	74-93-1	5188-07-8
Chemical Structure	H – S – H	$\begin{array}{c} \text{H} \\ \\ \text{H} - \text{C} - \text{S} - \text{H} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{H} \\ \\ \text{H} - \text{C} - \text{S} - \text{Na} \\ \\ \text{H} \end{array}$
Molecular Weight	34.08	48.11	70.08
Color	Colorless	Colorless	Colorless
Physical State	Gas	Gas	Liquid
Melting Point (°C)	-85.49	-123	-12
Boiling Point (°C)	-60.33	5.95	>210
Octanol/Water Partition Coefficient	0.96	0.78	-2.3
Density	1.539@0°C	0.8665@20°C	1.34@20°C
Odor	Rotten eggs	Rotten cabbage	Odorous
Odor threshold			
Water	0.000029 ppm	0.000024 ppm	Not determined
Air	0.0005 ppm	0.0016 ppm	Not determined
Water solubility @ 25°C	4.31 g/l (@20°C)	15.39 g/l	Miscible
Vapor Pressure (mmHg @ 22°C)	14469	1520	1.08E-6 (25°C)
Explosive limit	4.3 – 46%	3.9 – 22%	Not determined

b. Similar Metabolism

H₂S Metabolism

The metabolism of H₂S and MeSH appear to result in the same chemical species, sulfate (SO₄²⁻). H₂S enters the circulation directly across the alveolar-capillary barrier, where it dissociates in part, into the active sulfide ion (HS⁻). The most common route of exposure, and the one of most concern, is inhalation. The principle fate of absorbed H₂S following inhalation is oxidation to sulfates and excretion in the urine (Beauchamp et al, 1984). Most absorbed H₂S is oxidized by 15 hours following exposure (Kangas and Savolainen, 1987). Bartholomew et al. (1980) noted the primary location for these metabolic reactions was in the liver. H₂S can also be metabolized by methylation and reaction with metallo- or disulfide-containing proteins. However, the major route is oxidation of sulfide to sulfate (Beauchamp et al, 1984).

MeSH Metabolism

MeSH is a gas; therefore, the route of most concern is inhalation. The inhaled MeSH is rapidly absorbed and is readily oxidized to carbon dioxide and sulfate by splitting of the central carbon-sulfur bond. The primary end result is sulfate excreted in the urine (Blom et al, 1990).

Most of the MeSH metabolism work has been conducted following intraperitoneal (ip) injection (Derr and Draves, 1983;1984). These studies indicated that male Spague-Dawley rats eliminated 94% of the injected MeSH in the urine 21 hours after administration (Derr and Draves, 1983). MeSH is distributed in the plasma and in the blood cells (Al Mardini et al, 1988). Red blood cells are capable of oxidation of MeSH eventually to sulfate (SO₄²⁻) and formate (HCOO⁻) (Blom and Tagerman, 1988). The oxidation may also take place in the liver since MeSH is also a ligand for the mixed function oxidase (Dawson et al, 1983).

The 1990 Blom et al inhalation metabolism study with MeSH indicated that 80% of the administered MeSH was oxidized by red blood cells. Liver metabolism was not evaluated.

A recent study by Levitt et al. (1999) demonstrated that MeSH can be demethylated to H₂S, and further be converted to nonvolatile metabolites such as sulfate and thiosulfate in the cecal mucosa. Further studies by Furne et al. (2001) identified the same metabolic pathway for both H₂S and MeSH in other tissues including liver, plasma, and erythrocytes. Although cecal mucosa demonstrated a specialized function in metabolizing MeSH and H₂S, this data, as well as data obtained from other tissues demonstrate similar

metabolic profiles for MeSH and H₂S (Table 5, Figures 1 and 1a). Mazel et al. (1964) described a microsomal enzyme system that may play an important role in demethylation of MeSH to H₂S.

TABLE 5: Averaged Percent of Sulfur-Containing Metabolites During Incubation of Various Rat Tissue Homogenates with H₂S or MeSH

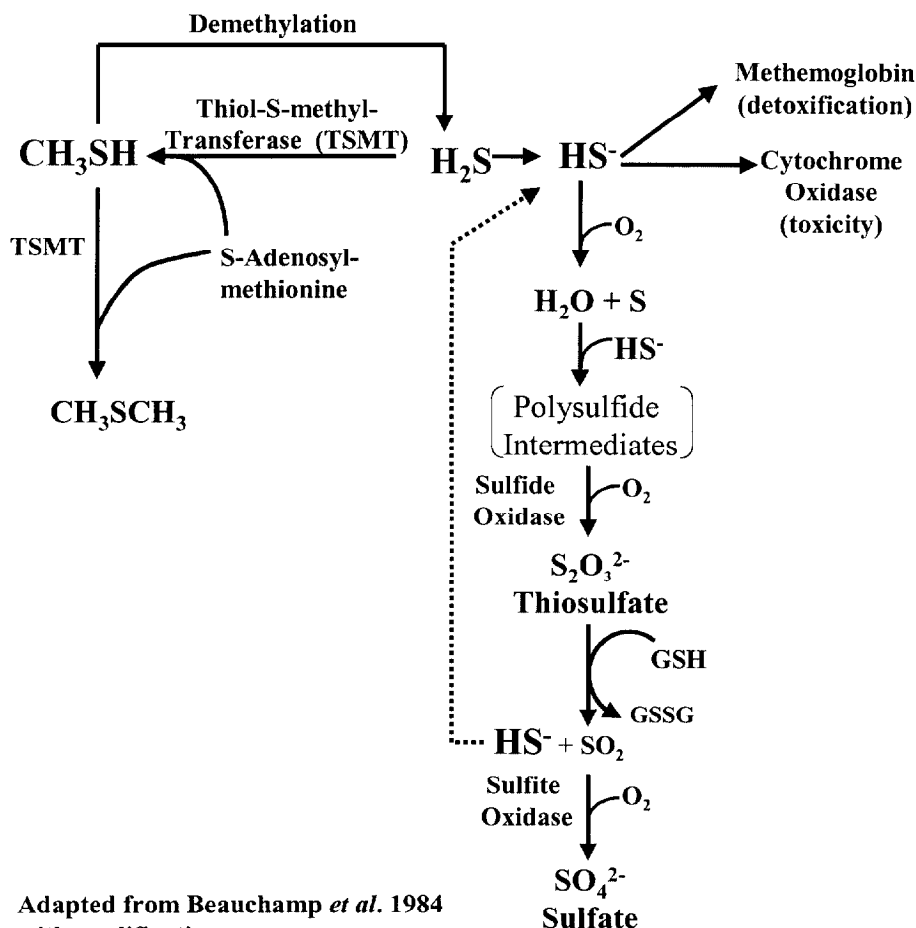
Tissue	H ₂ S			MeSH			
	ThioSO ₄	SO ₄	Total	ThioSO ₄	SO ₄	H ₂ S	Total
Liver	50	50	100	31	49	19	100
Muscle	60	40	100	31	52	<20*	100
Plasma	81	19	100	22	70	<20*	100
Erythrocytes	20	80	100	9	34	57	100

Adapted from Furne et al., 2001

Note: For muscle and plasma tissue treated with MeSH, levels of H₂S were below detection limits.

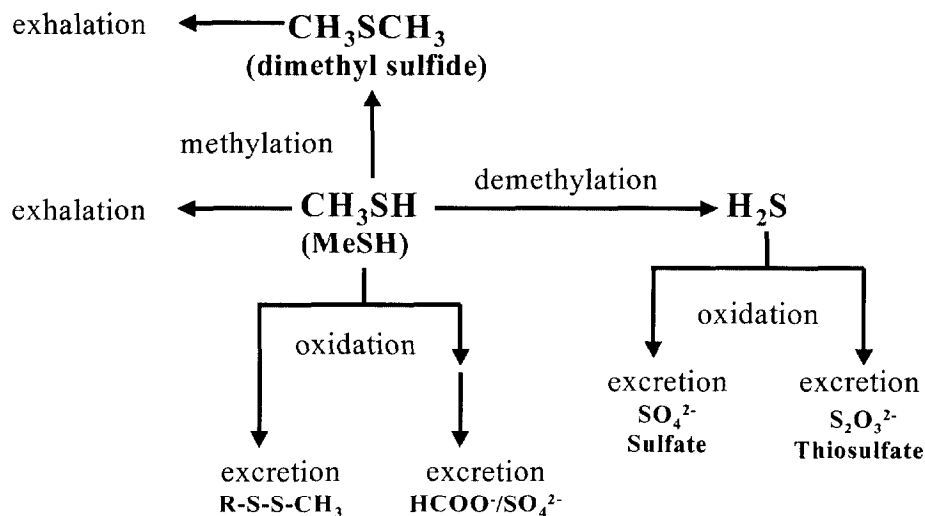
The exact pathway for MeSH metabolism has not been elucidated. It has strong similarities to H₂S in kinetics, primary route of elimination, and end product, sulfates (see Figures 1 and 1a).

FIGURE 1: Metabolic Scheme for H₂S



Adapted from Beauchamp *et al.* 1984 with modifications.

FIGURE 1a: Metabolic Schemes for MeSH



Adapted from Jappinen et al, 1993, with modifications

c. Similar Mechanism

The mechanism of toxicity of H_2S and MeSH is similar, i.e. cytochrome c oxidase inhibition. Waller (1977) reported that MeSH inhibits liver mitochondrial respiration by reacting with cytochrome c oxidase. This same mechanism of action has been reported for H_2S . Most investigators agree that MeSH acts like H_2S on the respiratory center, producing death by respiratory paralysis (Waller, 1977; Gosselin et al, 1984; Patty's, 1991). However, Wever indicated MeSH inhibitory activity for cytochrome c oxidase is much weaker than for H_2S (Wever et al, 1975). This indicates H_2S may be more toxic than MeSH.

d. Similar Acute Toxicity

The acute inhalation toxicity data summarized in Table 6 supports the statement that H_2S is more toxic than MeSH.

TABLE 6: Comparison of H₂S/MeSH/NaMeSH Toxicity Data

	H ₂ S (gas)	MeSH (gas)	NaMeSH (liquid)
Acute LC ₅₀ (ppm)	444 ¹	675 ¹	No data
Subchronic NOAEL'S (ppm)	Fischer-344 rats –80 ² Sprague-Dawley Rats- 30ppm (females), 80 ppm (males) ³ B6C3F ₁ Mice – 30 ⁴	57 ¹	No data

1 Tansy et al, 1981

2 CIIT 1983a

3 CIIT 1983b

4 CIIT 1983c

Symptoms associated with acute MeSH exposure are similar to those of H₂S. Inhalation of MeSH can cause narcosis, headache, nausea, pulmonary irritation, and convulsions in humans. Exposure to high concentrations can result in respiratory paralysis and death (Hazardous Properties, 1999).

e. Similar Subchronic Toxicity

The subchronic toxicity data are similar for H₂S and MeSH as reflected in the No Observed Adverse Effect Levels (NOAELS) shown in Table 6. When animals were exposed for 90 days to either chemical, no treatment – related changes were detected by gross or histopathological examination of the gut, lung, heart, liver, kidneys, or other organs. Body weights and organ weights were the only endpoints of overlap for the two chemicals for the 90-day studies. The results of these endpoints are summarized below.

Subchronic H₂S Exposures:

No treatment-related body weight changes were noted in male or female Fischer-344 rats exposed to airborne concentrations of 10, 30 and 80 ppm of H₂S for 6 hr/day, 5 days/wk for 90 days (CIIT, 1983a). However, when Sprague-Dawley rats were exposed to the same regimen, females at 80 ppm showed a significant (10%) decrease in body weight at the end of the study compared to controls. At 80 ppm, the body weight of male Sprague-Dawley rats was significantly less (8%) than controls during weeks 1-3, but the final body weight differences were not significant (CIIT, 1983b). Similarly, B6C3F₁ mice of both sexes exposed to 80 ppm, using the same testing regimen as above, showed a 7-14% decrease in body weight compared to controls (CIIT, 1983c).

Subchronic MeSH Exposures:

A 90-day repeat dose inhalation toxicity study has been conducted on MeSH. Male Sprague-Dawley rats were exposed to 2, 17, or 57 ppm MeSH for 7 hours/day, 5 days/week, for 90 days. Terminal body weights, organ weights, oxygen consumption, systolic blood pressure, intestinal transit activities and SMA 12/60 Analysis were evaluated. No mortality was observed in any of the sham or exposed population of rats. Average terminal body weights were lower in the exposed groups than those of sham controls for all rats. This difference was only statistically different at the 57 ppm exposure level, which showed a 15% decrease in terminal body weight. The authors state that although some average organ weights were significantly different from corresponding sham values, there were no obvious dose-related trends (Tansy et al, 1981).

Statistically, significant changes were observed in serum components of blood samples from animals of all exposed groups. However, none of these trends were dose-related at the 95% confidence level. The H₂S study evaluated blood cell parameters, but did not evaluate serum components.

f. Conclusion of Data Comparison

In conclusion, the data presented for MeSH and H₂S indicates they have similar physical/chemical characteristics, similar metabolic profiles, similar mechanism, and similar toxicity following acute and subchronic exposure. The toxicity of H₂S is slightly greater than MeSH as judged by several authors (Tansy et al, 1981; Patty's 1991). This may be due to the higher affinity H₂S has for the cytochrome c oxidase enzyme than does MeSH as explained by Wever (1975). Based on this information, the use of the H₂S data to fill the Reproductive/Developmental data gap should be accepted as a worse case scenario.

2. H₂S Reproductive/Developmental Neurotoxicity Study

In 2000, Dorman et al., published a reproduction/developmental toxicity study with H₂S. This study was conducted using the OECD 421 guideline and included a neurodevelopmental component. Briefly, Sprague-Dawley rats were exposed via inhalation to concentrations of H₂S up to 80 ppm. The data from this study indicate H₂S does not cause adverse effects on reproductive endpoints, or on developmental endpoints including: pinnae detachment, incisor eruption, negative geotaxis, eyelid separation, vaginal patency, or balano-preputial separation. In addition, no effects were observed in motor activity, passive avoidance, functional observation battery, acoustic startle response or neuropathology (including gross and histological brain pathology). In conclusion, this study indicated that H₂S

is neither a reproductive toxicant, teratogen nor a behavioral developmental neurotoxicant in the rat at levels significantly higher than occupationally relevant exposure concentrations (e.g. 10 ppm TWA, ACGIH).

The available data are sufficient to assess the reproductive/developmental hazard of MeSH and NaMeSH.

SUMMARY: No additional testing is proposed for purposes of the HPV Challenge Program.

Additional Concern: MeSH is highly odorous (odor threshold 1.6 ppb). Therefore, it has excellent warning properties. People do not willingly or unknowingly expose themselves to this chemical at concentrations greater than 1-10 ppm. The odor does preclude testing MeSH at high concentrations because communities surrounding the contract laboratories complain about the nuisance odor.

X. CONCLUSIONS

Sufficient data to evaluate several of the endpoints listed in the HPV Challenge are available for MeSH and NaMeSH as summarized in Table 7.

Physical/chemical characteristics (melting point, boiling point, vapor pressure, and water solubility) are available or can be calculated for MeSH and NaMeSH.

To evaluate environmental fate, data are available or can be calculated using EPA approved models for photodegradation and fugacity (transport and distribution in the environment).

NaMeSH is toxic to daphnia, similar results are expected for MeSH. No adequate data are available to assess the toxicity to fish and alga. Therefore, acute fish toxicity and algae inhibition studies are proposed for NaMeSH.

Mammalian toxicology data on MeSH and NaMeSH have shown central nervous system effects following acute exposure to high doses. Adequate data are available indicating MeSH and NaMeSH are not genotoxic. Since MeSH and NaMeSH are similar to H₂S, adverse effects to the reproductive system or developing fetus are not anticipated. Repeated exposure to MeSH and NaMeSH is not expected to cause adverse effects based on the results from a 90-day inhalation study with MeSH.

SUMMARY: EPIWIN software is proposed to estimate photodegradation and fugacity for MeSH and NaMeSH. In addition, acute fish toxicity and algae inhibition studies are proposed with NaMeSH.

TABLE 7: Matrix of Available Data for MeSH and NaMeSH by OECD SIDS Endpoints

OECD SIDS Endpoints	Methyl Mercaptan (MeSH)	Methyl Mercaptide (NaMeSH)
Physicochemical		
Melting point	Data available	Data available
Boiling point	Data available	Data available
Vapor Pressure	Data available	Data available
Water Solubility	Data available	Data available
Octanol/Water Partition Coefficient	Data available	Data available
Environmental Fate		
Biodegradation	Data available	Data available
Photodegradation	Calculated	Calculated
Hydrolysis	NA based on chemical properties	NA
Fugacity	Calculated	Calculated
Aquatic Toxicity		
Algae	RA	Testing Proposed
Invertebrate	RA	Data available
Fish	RA	Testing Proposed
Acute Mammalian Toxicity		
Oral	N/A based on chemical properties	Data available
Inhalation	Data available	NA
Dermal	NA	Data available
Repeated Dose Toxicity		
Inhalation	Data available	RA
Genetic Toxicity		
Point Mutation	RA	Data available
Chromosomal Effects	Data available	Data available
Reproductive Toxicity		
Inhalation	RA – H ₂ S	RA – H ₂ S
Developmental Toxicity		
Inhalation	RA – H ₂ S	RA – H ₂ S
Key: NA – Not Applicable RA – Read Across From Existing Data or From Proposed Testing		

XI. REFERENCES

- Al Mardini, H., Bartlett, L.J., and Record, L.S., CO., 1988. Effect of methionine loading and endogenous hypermethioninemia on blood mercaptans in man. *Clin. Chim. Acta* 176:83-90.
- ACGIH, 2001. Guide to occupational exposure limits.
- Bartholomew, T.C., Powell, G.M., Dodgson, K.S., et al., 1980. Oxidation of sodium sulphide by rat liver, lungs and kidney. *Biochem. Pharmacol.* 29:2431.
- Beauchamp B.O., Bus J.S., Popp J.A., Boreiko C.J., and Andjelokivish D.A., 1984. A critical review of the literature on hydrogen sulfide toxicity. *Crit. Rev. Toxicol.* 13:25-97.
- Blom, H. S., Chamuleau, R., Rothuizen, J., Deutz, N., and Tangerman, A., 1990. Methanethiol Metabolism And Its Role in the Pathogenesis of Hepatic Encephalopathy in Rats and Dogs. *Hepatology*, 11(4):682-689.
- Blom, H.J. and Tangerman, A., 1988. Methanethiol metabolism in whole blood. *J. Lab Clin. Med.* 111:606-610.
- CIIT a., 1983. Toxigenics, Inc. 90-Day Vapor Inhalation Toxicity Study of Hydrogen Sulfide in Fischer 344 Rats, Vol. 1 and 2. CIIT Docket No. 22063. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.
- CIITb *ibid.* "in Sprague – Dawley Rats".
- CIITc *ibid.* "in B6C3F1 mice".
- CRC Handbook of Chemistry and Physics, 1995, 76th Edition, page 731.
- Dawson, J.H., Andersson, L.A. and Sono, A., 1983. The diverse spectroscopic properties of ferrous cytochrome P-450-CAM ligand complexes. *J. Biol. Chem.* 258:13637-13645.
- Derr, R.F. and Draves, K., 1984. The time course of methanethiol in the rat. *Res. Comm. Chem. Path. Pharma.* Vol 46(3): 363-369.
- Derr, R.F. and Draves, K., 1983. Methanethiol metabolism in whole blood. *J. Lab. Clin. Med.* Vol. 39(3):503-506.
- Dorman, D.C., Brenneman, K.A., Struve, M. F., Miller, K.L., James, R. A., Marshall, M. W., and Foster, P.M.D., 2000. Fertility and Developmental Neurotoxicity Effects of Inhaled Hydrogen Sulfide in Sprague-Dawley Rats. *Neurotoxicology and Teratology*, 22:71-84.

Elf Atochem, 1994. Acute dermal toxicity in rats. CIT. Unpublished study.

Elf Atochem, 1989. Acute oral toxicity in rats. CIT. Unpublished study.

Elf Atochem, 1997. Bone marrow micronucleus assay in male and female Swiss Webster mice following acute nose only exposure to methyl mercaptan. SRI International. Unpublished study.

Elf Atochem, 1999. Bone marrow micronucleus test by oral route in mice with sodium methylmercaptide. CIT. Unpublished report.

Elf Atochem. Sodium methyl mercaptide. Reverse mutation assay by Ames test. CIT. Unpublished study. 1992.

Elf Atochem, 1995. In vitro mammalian chromosome aberration test in cultured human lymphocytes with sodium methylmercaptide. CIT. Unpublished study.

EPIWIN, 2001. Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY, USA.

Furne J., Springfield J., Koenig T., DeMaster E., and Levitt M.D., 2001. Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. *Biochem. Pharmacol.* 62:255-259.

Gosselin, R. E., Smith, R. P., and Hodge, H. C., 1984. Clinical Toxicology of Commercial Products, 5th ed., Section II, Ingredients Index, pp. 115-116. Williams and Wilkins, Baltimore.

Pradyot Patnaik, ed. 1999. Hazardous properties of chemical substances. 2nd Ed. John Wiley & Sons, Inc. New York. Sulfur-containing organics (miscellaneous).

Jappinen, P., Kangas, J., Silakoski, L. and Savolainen H., 1993. Volatile metabolites in occupational exposure to organic sulfur compounds. *Arch. Toxicol.* 67:104-106.

Kangas, J. and Savolainen H., 1987. Urinary thiosulfphate as an indicator of exposure to hydrogen sulphide vapour. *Clin. Chim. Acta* 164(1):7-10.

Lange's Handbook of Chemistry, 1985. 15th Edition, Table 8.8.

Levitt M.D., Furne, J., Springfield J., Suarez F., and DeMaster E., 1999. Detoxification of hydrogen sulfide and methanthiol in the cecal mucosa. *J. Clin Invest.* 104(8):1107-1114.

Mazel P, Henderson J.F., Axelrod J., 1964. S-Demethylation by microsomal enzymes. *J. Pharmacol Exp Ther.* 143:1-6.

Patty's Industrial Hygiene and Toxicology, 1991. eds. G. Clayton and F. Clayton. 4th Ed. IIF:4314-4317.

Tansy, M. F., Kendall, F. M., Fantasia, J., Landin, W. E., and Oberly, R., 1981. Acute and Sub chronic Toxicity Studies of Rats exposed to Vapors of Methyl Mercaptan and other Reduced-Sulfur Compounds. *J. Tox. Environmental Health*. 8:71-88.

US EPA, 1999. The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. OPPT, EPA. Washington, DC., USA.

US EPA, 1999. Determining the Adequacy of Existing Data. OPPT, EPA. Washington, DC., USA.

Waller, R. L., 1977. Methanethiol Inhibition of Mitochondrial Respiration. *Tox. Appl. Pharm.*, 42:111-117.

Wever, R., Van Gelder, B. F., and Dervartanian, D. V., 1975. Biochemical and Biophysical Studies on Cytochrome c Oxidase. XX. Reaction with Sulphide. *Biochim. Biophys. Acta*. 387:189-193.